DNA sequence, while using codons which are favorable for expression in E.coli. Restriction enzyme sites were added on both ends. The synthesized DNA sequence was cloned into an expression vector. The protein was bacterially expressed and purified to homogeneity using reverse-phase HPLC. Protein identity was verified using MALDI-TOF mass spectroscopy. Near UV circular dichroism spectroscopy confirmed the strongly alpha helical nature of the protein. Guanidinuium chloride denaturation showed that the single chain four-alpha-helix bundle protein is twice as stable as the dimeric dihelical protein. The sigmoidal character of the unfolding reaction was conserved, the sharpness of the transition increased. Our single chain four-alpha-helix bundle protein bound halothane with a dissociation constant of 1.2 mM, as shown by tryptophan fluorescence quenching. This single chain four-alpha-helix bundle protein can now be used as a scaffold to incorporate natural membrane protein sequences to examine general anesthetic interactions in detail.

2989-Pos Board B94

Binding Profiles Based on Normal Mode Analysis as a Foundation for a Unified Approach to Allosteric Activation of Prolactin Receptor

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Two of the most fundamental biological processes are ligand binding and allosteric signaling. Despite their (often) direct linkage, a unified model of the underlying dynamics is not well established. The harmonic motions identified by normal mode analysis (NMA) provide a natural coordinate system for conformation space. Binding profiles and allosteric profiles based on NMA allow for a unified model which describes the propensity for various structure motions to promote/inhibit binding and allosteric activation. The authors have recently developed the allosteric model; the current work presents the binding model.

The normal modes describe local motions accessible to a conformation. Each of the modes is followed over a small displacement, leading to a set of new conformations. This procedure is repeated to generate an ensemble, where each conformation is defined by its sequence of normal modes. Ensembles are generated around a receptor and ligand. Each receptor conformation is paired against each ligand conformation and scored for: (1) compatibility of interface shape; and (2) compatibility of interface dynamics. These scores are attributed to the normal mode sequences associated with each candidate conformation. The scores are summed over all possible conformation pairings, producing a binding profile that defines each normal mode's propensity to contribute to compatible interface shape and interface dynamics.

The prolactin receptor dimerizes and preferentially binds prolactin at one binding site, and then binds another prolactin at the second binding site - activating the receptor. Binding profiles are generated for prolactin and prolactin receptor, isolated from the various complexes along the activation pathway. The goal is to identify the dynamics that regulate binding site preference and allosteric activation. Future work will incorporate NMR studies to validate and refine the initial results.

2990-Pos Board B95

Solid-State NMR Study of Ligand Binding to Human Peripheral Cannabinoid Receptor CB2

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The peripheral cannabinoid receptor CB2 belongs to the family of G protein coupled receptors (GPCRs). Ligand binding studies on CB2 are complicated by high affinity of the endogenous or exogenous cannabinoid ligands for the lipid matrix that hosts the GPCR. Here we show that solid-state NMR distinguishes between specific ligand-binding to CB2 and nonspecific interactions with the lipid matrix. Experiments were conducted with recombinant, human CB2 expressed in *E. coli*, purified and functionally reconstituted into unilamellar liposomes. Location, structure, and dynamics of ligands in the lipid matrix were probed by NMR as well as neutron diffraction. The synthetic agonist CP-55,940 locates near the hydrophobic/hydrophilic interface of bilayers with its bond linking hydroxyphenyl and hydroxycyclohexyl rings perpendicular to the bilayer normal, while the endogenous agonist 2-AG orients parallel to the bilayer normal with the glycerol moiety near the hydrophobic/hydrophilic interface and the arachidonoyl chain in the hydrophobic region. Both ligands maintain a high level of conformational flexibility and have lateral diffusion rates in membranes comparable to those of lipids. Ligand binding to CB2 drastically shortens transverse relaxation times of the ^{2}H-labeled ligands which distinguish between specific and non-specific binding events. Competition binding experiments with protonated and selectively deuterated CP-55,940 showed that ~90% of the reconstituted CB2 was ligand-binding competent and formed a one-to-one complex with the ligand. Activation of G protein by agonist-bound CB2 was confirmed in a G protein activation assay. The endogenous 2-AG has a binding affinity to CB2 that is orders of magnitude lower compared to CP-55,940. The possibility that cannabinoid ligands approach the receptor from the lipid matrix will be discussed. Experiments are underway to gain structural insights into specific binding interactions based on selective isotope-labeling of both ligand and recombinant CB2.

2991-Pos Board B96

Fluorescence Guided Force Microscopy (FGFM) Used to Measure Receptor Ligand Interactions in Live Mammalian Cells

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Fluorescence Guided Force Microscopy (FGFM) is a new technique that was developed in order to measure and quantify ligand surface interactions on the cell surface in living cells. Using this method we can determine the binding affinity of ligands with surface receptors and their spatial distribution by combining force volume measurements, confocal microscopy and atomic force microscopy into one new instrumental setup. In order to validate this new method we covalently linked a ligand, Bone Morphogenetic Protein 2 (BMP2) to an Atom Force Microscopy (AFM) probe and acquired topographical and force binding information. To visualize caveolae by confocal microscopy, membrane domains known to co-localize with BMP receptors on the cell surface, Caveolin-1 was fused to green or red fluorescent protein and used as a marker. We transfected C2C12 cells with plasmids encoding Caveolin-1 isoforms alpha and beta (fused to green fluorescent protein) and Caveolin-1 alpha (fused to red fluorescent protein). Detecting the unbinding forces, surface topography and fluorescent protein location on the live cell surface, required combining Force Volume measurements, AFM and confocal imaging; which we achieved through the integration of the Veeco Bioscope2 AFM module, equipped with a closed loop scanner and a Zeiss LSM510 NLO confocal. The collection of high resolution confocal images, AFM images and unbinding force curve data on live cells allowed us to resolve the spatial distribution of binding events on the plasma membrane of C2C12 cells. This data showed that BMP 2 bound with the highest affinity inside Caveolin-1 isoforms.

2992-Pos Board B97

Nanoparticles Masquerade as "Self" to Inhibit Phagocytosis

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A major challenge for injecting particles or implanting biomaterials into the body is that they activate immune cells such as macrophages, the cells that normally function to clear invading pathogens. Interestingly, macrophages have a surface receptor mechanism which prevents them from phagocytosing our own "self" cells. During initial macrophage engulfment, macrophages recognize foreign and self targets because they both have antibodies or plasma complement proteins on their surface. However, before the macrophage engulfs the target, self cells are checked for the presence of the surface protein CD47 which will bind to the macrophage receptor SIRPα (CD172), and we show that CD47-SIRPα interactions in cell-cell adhesion, with human macrophages in sparse culture, produce phagocytosis inhibition. Whether the CD47 interaction is functional with small targets of phagocytosis is unclear and relevant perhaps to nano-sized targets. We show that CD47 coupled to a series of synthetic beads can inhibit uptake by macrophages. However, we need to test this interaction and observe how these results are consistent with in vivo systems. We are currently testing whether nanoparticles that have surface immobilized hCD47 or a portion of it will not be phagocytosed in nobese diabetic (NOD) mice, we believe that hCD47 will bind to this mSirpa strain and that could bring a better understanding of the interaction at a nanoscale.

2993-Pos Board B98

In Vivo Binding Kinetics and Stoichiometry of Toll-Like Receptor 9 and CpG DNA Resolved by Multiparametric Single Molecule Techniques

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Toll-like receptor 9 (TLR9) activates the innate immune system in response to oligonucleotides rich in CpG whereas DNA lacking CpG could inhibit its activation. Although in vitro experiments demonstrate TLR9 binding to nucleic acids, the mechanism of how this receptor interacts with nucleic acid and becomes activated in live cells is far from behind understood. Here, we report on the successful implementation of single molecule tools, constituting fluorescence correlation spectroscopy (FCS), fluorescence cross-correlation spectroscopy (FCCS), photon count histogram (PCH) and fluorescence lifetime imaging (FLIM) to study the interaction of TLR9-GFP with Cy5 labeled oligonucleotide containing CpG or lacking CpG in live cells.